PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference WPP95905	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/GB2007/000488	International filing date *(day/month/year)* 12.02.2007	Priority date *(day/month/year)* 10.02.2006
International Patent Classification (IPC) or national classification and IPC INV. C12N15/85		
Applicant Oxitec Limited		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 11 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
2007-12-10	05.05.2008
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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2007/000488

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-85 as originally filed

Sequence listings part of the description, Pages

1-166 as originally filed

Claims, Numbers

1-44 as originally filed

Drawings, Sheets

1/59-59/59 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

5. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 70.2 (e)).

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-18, 23, 25-36
	No:	Claims	19-22, 24, 37-44
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-44
Industrial applicability (IA)	Yes:	Claims	1-44
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ on paper

   ☒ in electronic form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in electronic form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment* on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

1. Additional remarks to item V (reasoned statement under Rule 66.2(a) (ii) with regard to novelty, inventive step or industrial applicability)

1.1 Present application

The present application is directed to a gene expression system comprising splice control sequences. The splice control sequence allows an additional level of control of gene expression by providing a mechanism for alternative splicing in a claimed sex, stage, germline or tissue specific manner. The gist of this application is the sex-controlled expression of lethal genes for the control of an organism population. In other words an heterologous lethal gene is expressed in one sex and not the other by sex specific alternative splicing of the lethal gene. Examples of splice control sequences are sequences derived from tra or dsx intron retaining the alternative splicing function.

1.2 Prior art documents

The present communication refers to the documents cited in the International Search Report (ISR). Said documents are numbered as in the ISR, i.e. D1 corresponds to the first document cited in the ISR. The numbering will be adhered to in the rest of the procedure.

These prior art documents disclose, among other, the following data:

(i) **D1** corresponds to a prior Applicant's application directed to the development of expression systems for insect pest control. These polynucleotide expression systems are characterized by comprising a gene to be expressed and its promoter, where a product of the gene to be expressed serves as a positive transcriptional control factor for the promoter and the product or its expression is controllable. Exemplified are systems comprising tTA gene product combined with tetO operator whose expression is regulated by tetracycline, or a tTA variant denominated tTAV constitutively highly expressed (p.18). Interestingly, example 12 provides pLA1188 (SEQ 22, Fig.17) where Cctra intron within tTAV ORF allows reconstitution of intact tTAV by alternative splicing of intron only in medfly female larvae. According to paragraph 3 of page 42, female larvae yielded PCR products corresponding to the expected sizes that would result from splicing in the pattern of the endogenous Cctra gene. The authors conclude that the Cctra intron can splice correctly in a heterologous context and provides a suitable method for introducing sex-specificity into a positive feedback construct. Thus combination of non sex-specific expression of a transcriptional activator with sex-specific

expression through splicing, of a functional RNA under the transcriptional control of the transcriptional activator allows sex-specific expression of a target gene (Figure 18 and p.13, last paragraph - p.14, paragraph 3).

(ii) **D2** is directed to the identification of the cis-element which is required for the sex-specific splicing of dsx in tissues of Bombyx mori (Bm). It reports that a Bm mini gene consisting of exon 1 and 5 and shortened introns 2 and 4 contains the information necessary for the correct regulation of alternative splicing.

(iii) **D3**studies the molecular mechanisms of sex differentiation in the mosquito Anopheles gambiae with the goal of identifying genes for inducing specific male sterility or for sex-controlled expression of lethal genes. Dsx gene is shown to have a role in determining the sexual fate in A. gambiae. D2 also reports the presence of regulatory elements in the 3' UTR of the female-specific exon (Agdsx RE elements), which display high homology with the Dmdsx splice enhancer dsxRE elements and the Dmfru RE elements (Table 2). These Agdsx RE elements are located much further downstream from the 3' splice acceptor site that in Drosophila, a situation similar to Dmfru RE elements. The authors conclude that the identification of female and male specific transcripts of Agdsx represents an important step towards the understanding of the sex differentiation process in A. gambiae and will facilitate the development of genetic tools to manipulate sex ratios in mosquitoes, for example by inducing the sex-specific splicing of a dominant lethal gene.

(iv) **D4** discloses the identification of an Actin-4 promoter as a female specific promoter in Aedes aegyptii. Said promoter is considered useful for the preparation of transgenic strains of mosquitoes carrying dominant conditional-lethal genes.

(v) **D5** reports the development of plasmid carrying tetracycline repressible transactivator as a transactivator and lethal gene for the control of insect pests population (Sterile Insect Technique), in particular medfly (Med. fruitfly). The results shown that highly efficient, repressible, dominant lethality can be achieved in the medfly using these compact expression systems which give performance characteristics appropriate for incorporation into SIT based control programs. Said system is expected to work across a wide phylogenetic range (easier transfer to other species) because of the absence of

tephritid DNA.

(vi) **D6** corresponds to the late publication of main examples of the current application.

1.3 Statement with regard to novelty and inventive step (Articles 33 (2) and (3) PCT)

The arguments of the Applicant, given in his letter dated 10.12.2007 responding to the Written Opinion of the ISA, were taken into consideration when establishing this report, but are considered not to be relevant for the objections presented below.

1.31 Novelty

The subject-matter of claims **1-18, 23, and 25-36** does not meet the requirements of Articles 33 (2) and (3) PCT, because said claims lack novelty in view of D1 and/or their lack of clarity in the sense of Article 6 PCT.

The plasmid pLA1188 (Figure 17, SEQ ID N°22) disclosed in example 12 of D1, corresponds to an expression system for tTAV ORF which comprises an Cctra intron. It was injected into Medfly embryos and products corresponding to the expected sizes that would result from splicing in the pattern of the endogenous Cctra gene were obtained as identified by PCR analysis. The authors of D1 - the present Applicant - then conclude in paragraph 3 of page 42 that these data indicate that the Cctra intron can splice correctly in a heterologous context. As a consequence a novelty objection was raised over D1 in the written opinion.

The Applicant argued in his letter dated 10.12.2007 that D1 is not prejudicial to novelty because no functional protein was actually obtained in D1. This Authority respectfully disagrees. It is first pointed out that the subject-matter of claims 1-37 is a product claim, and not a method claim. The fact that the use of the claimed polynucleotide expression system allows the expression of a functional protein is not an essential feature of the system per se, because said system does not comprise a protein. As outlined in the PCT Guidelines, Part II, Chapter 5.21, a product claim defined for a particular use is construed as meaning a product suitable for the stated use. Since D1 reports that said intron allows reconstitution of intact tTAV by alternative splicing of intron in medfly female larvae only (sex-specific manner) (see § 1.2 i), D1 plasmid pLA1188 is

considered to be suitable for the sex-specific expression of tTAV. With regard to claim 13, homologues of a specific promoter is considered to embrace any promoter (Article 6 PCT). D1 therefore anticipates novelty of **claims 1-6, 8-18, 23 and 25-36**.

For sake of completeness it is also pointed out that the expression "functional protein" is not clear in the sense of Article 6 PCT, because the function of the protein is not identified. In particular almost any protein is functional in terms of immunogenicity. Thus it cannot be excluded that the among the several tTAV protein actually obtained in D1, which, according to the present specification, results from an induced frame shrift in the transcript leading to the removal of 4 nucleotides, is immunogenic, and as such functional.

1.32 Inventiveness

Should the above novelty objection surprisingly be overcome, the subject-matter of **claims 1-44** is also not allowable because of lack of an inventive step, pursuant to Article 33 (3) PCT, for the reasons outlined below.

(i) As outlined throughout the specification, the problem to be solved by the present application is to provide an alternative expression system for insect pest control.

Given the positive results obtained in D1 in terms of sex specific alternative splicing, the skilled person faced by the above problem would expect to solve it using the plasmid pLA1188 taught in D1. This reasonable expectation of success is confirmed by D1 own conclusions that (1) the Cctra intron can splice correctly in a heterologous context, providing a suitable method for introducing sex-specificity into a positive feedback construct and (2) this work provides a general method for manipulating the expression of genes using other sex-specific introns known in the art (p.42, last paragraph).

The Applicant, which is the Applicant of D1, now argued in the present specification (pages 29-30) and in his reply to the WO-ISA, that D1 does not solve the problem of the present application. He stated that the Cctra intron used in PLA1188 excised additional nucleotides, what resulted in an induced frame shift in the transcript and the removal of four nucleotides of tTAV sequence in the female-specific transcript. As a consequence none of the alternatively spliced transcripts obtained with the system of D1 were capable

of encoding a functional tTAV protein and D1 was indeed not capable of solving the above problem.

This line of argumentation cannot be followed. In the absence of any concrete data, neither in the specification, nor within the Applicant's reply to the WO-ISA, supporting the allegation that D1 does not work, there is no evidence that a technical problem actually exists in D1. The lack of inventive step raised in the written opinion is therefore maintained (see § 1.32 of the WO-ISA).

The polynucleotide expression systems of present claims 19-22, 24 and 27 differ from the system disclosed in example 12 of D1 by the presence of a different splice control sequence or a different effector gene (RNAi). D1 conclusion however provides a clear incentive to try alternatives of the Cctra intron. By doing so the skilled person would inevitably start from insect genes, whose expression is known to be sex specific, such as the dsx genes from Drosophila or Aedes gambiae whose RE elements have already been studied (see D2 and D3, § 1.2 ii and iii respectively) or Actin-4 gene known for comprising a female specific promoter (see D4, § 1.2 iv). Thus, **claims 19-22 and 24** are considered as obvious alternatives for the person skilled in the art trying to solve the above technical problem, and, as such, do not involve an inventive step.

The same objection is raised against inventiveness of **claims 37 and 38-44** because the purpose of D1 is to use its expression system for the control of insect population (see § 1.2 i). It also already teaches the use of RNAi as an effector gene in said expression systems (D1: claim 22). No inventive step can be acknowledged for the claims 1-44 on file.

(ii) The attention of the Applicant is further drawn to the objection of lack of clarity and inventive step raised in section § 2.1 of the WO-ISA, which is maintained herein. This objection is merely based on the lack of clear technical features of the "splice control sequences" referred to throughout the claims. It is immediately visible that, despite the objection raised previously, these essential components of the claimed system are only defined by the desired result, namely the sex and/or stage and/or germline and/or tissue specific alternative splicing of the heterologous polynucleotide present in the system when expressed in a organism. The claims on file state the technical problem to be

solved, without indicating, in terms of technical features, how this problem is solved. The claims therefore also lack clarity in the sense of Article 6 PCT and inventive step in the sense of Article 33 (3) PCT, because the solution to a problem cannot be the problem itself.

The Applicant argued that it is appropriate to define this technical feature in a way that hints as its function as it is done for a "promoter", "enhancer" or "activator" and outlined that ample example of splice control sequence are provided. This Authority respectfully disagrees. The wording "promoter", "enhancer" etc... is allowable in the sense of Article 6 PCT, if any promoter, enhancer is suitable for the desired purpose. The Applicant's own allegation that the splice control sequence selected in D1 does not permit to obtain the desired result, namely the obtention of a "functional" tTAV protein, is a clear indication that all "splice control sequence" do not permit to get the desired result. This confirms that the claims on file attempt to define the subject-matter in terms of the result to be achieved, what is not allowable (see also § 2.1 below).

2. Certain observations on the international application (Articles 5 and 6 PCT)

2.1 Lack of clarity

It is clear from the specification that the "splice control sequence" is the key component of the expression system presently claimed. However said essential feature is only characterized by being "capable of mediating" alternative splicing which should be sex, stage, germline and/or tissue specific in some conditions ("in cooperation with a spliceosome"). Thus the reference to "splice control sequence" corresponds to a definition by the result to be achieved (see § 1.32 ii above), what is not allowable in the sense of Article 6 PCT (PCT Guidelines, Part II, Chapter 5.35). This lack of clarity leads to a lack of inventive step as outlined in section § 1.32 (ii) above. Any independent claim must contain all the technical features essential to the definition of the invention. In particular the essential technical features of the claimed expression system as compared to D1 possibly leading to a different result should be included in the claims.

The indication of the source of a sequence ("derived from ...") does not limit the scope of the claims 15-22. Actually the source of a sequence is interpreted as a "product by process" feature which is not clear in the sense of Article 6 PCT, because the same sequence may be obtained from different species or different strains of the same

species as confirmed by the consensus sequences already proposed for dsx gene RE elements (see, for example, D3, § 1.2 iii).

2.2 Lack of technical support

The above objection for lack of clear technical features leads to an objection for lack of technical support over the whole of the broad field claimed, pursuant to Articles 6 and 5 PCT (PCT Guidelines, Part II, Chapters 5.43-5.45). Present claims embrace the expression of the heterologous polynucleotide in any organism, i.e. in other words humans, bacteria, plants etc ..., whereas the "splice control sequences" referred to in the application are all derived from a few insect genes whose expression is sex specific. Which spliceosome should be present in the "organism" to ensure the alternative splicing mediated by Cctra intron?